Indonesian Journal of Cancer Chemoprevention, June 2017
ISSN: 2088–0197
e-ISSN: 2355-8989

Acetoxy Chavicol Acetate (ACA) Concentration and Cytotoxic Activity of *Alpinia galanga* Extract on HeLa, MCF7 and T47D Cancer Cell Lines

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Abstract

Due to severe side effect and non-specific chemotherapeutic agent, screening and discovery for cancer therapy are still working, especially from natural resources. Traditionally, people used herbal medicine either to prevent or cure diseases. One of herbal that commonly used in Indonesia is *Alpinia galangal*. Previous study stated that active compound is acetoxy chavicol acetate (ACA) and active as anticancer. This research aimed to determine ACA concentration and cytotoxic activity of *Alpinia galanga* extract (AGE) from three local markets on HeLa, MCF7 and T47D cell lines. The galangal used from three local markets namely Pasar Legi Surakarta, Beringharjo Yogyakarta, and Wonogiri. The extraction was performed by maceration using 96% ethanol as solvent. ACA quantitation using UV spectrophotometer at λ = 208.5 nm. Samples were prepared by liquid-liquid extraction using an ethyl acetate. Cytotoxic activities were performed by MTT assay. The result showed that the concentration of ACA of AGE from the three local markets were 3.798; 0.035; and 0.009 % w/w, respectively. Cytotoxic activity, describes as IC_{50} value, on HeLa cell line of AGE from three local markets, in order were 13.26; 36.32 and > 100 µg/ mL. Meanwhile, AGE from Pasar Legi on MCF7 and T47D cell lines have IC_{50} value of 15.80; 12.50 µg/ mL, respectively. In contrast, two other samples have IC_{50} values of greater than 100 µg/ mL. The highest activity was from the highest concentration of ACA on the samples.

Keywords: *Alpinia galanga*, HeLa, T47D, MCF7 and Acetoxy chavicol acetate

INTRODUCTION

Prevalence of cancer incidence is quite high and the second cause of death (13%) after cardiovascular diseases. The leading cancer in Indonesian women are breast and cervical cancers (Kemenkes RI, 2016). In Indonesia, 2013, the prevalence of breast cancer is ranked second after cervical cancer (n=61,682) (Kemenkes RI, 2015). Therapies that can be done for cancer patients include surgery, radiotherapy, targeted therapy, and chemotherapy. The action mechanism of inhibition of chemotherapeutic agents are inhibit cell proliferation and induction of apoptotis. However, high side effects and resistance from chemotherapeutic drugs, are encouraging the use of traditional herbal medicine as alternatives to cure cancer (Ma'at, 2004).

Galangal (*Alpinia galanga*) is a Zingiberacea family plant, which contains phenylpropanoid compounds such as 1'-acetoxychavicol acetate, 1'-acetoxyeugenol acetate, trans-p-cumaril diacetate, 1'-hydroxyccapsol acetate and trans-p-cumaril alcohol (Matsuda, 2005). The compound of 1'-acetoxy chavicol acetate has been reported to have cytotoxic activity against breast cancer cells (MCF-7) (Chauhan, *et al.*, 2014). The cytotoxicity value of IC_{50} obtained by compound 1'-acetoxy chavicol acetate on MCF7 cells was 23.9 µM (Lee and Houghton, 2005). The galangal extract containing 1'-aceto-chavicol acetate compound have cytotoxic activity by inhibition of cell proliferation in G0-G1 phase with IC50 20 µM (Hasima, *et al.*, 2010). This study was conducted to determine the cytotoxic activity of galangal extract from three local markets on various cancer cell lines.

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MATERIALS AND METHODS

Materials

Alpinia galanga rhizomes were obtained from three local markets, Pasar Legi Surakarta, Wonogiri, Beringharjo Yogyakarta. HeLa, T47D and MCF7 cell lines from the Cytotoxic Laboratory of Faculty of Pharmacy UMS. Ethanol 96%, hexane, ethyl acetate, chloroform, aquadest, standard solution 1'-acetoxy chavicol acetate, DMEM culture medium, FBS, penicillin-streptomycin, PBS solution, Tripsin-EDTA, DMSO, MTT assay solution, SDS 10% in 0.1N HCl, aluminum foil, UV spectrophotometer Mini.

Methods

Extraction was done by maceration using ethanol 96% and the extract was partitioned using ethyl acetate. The top layer (ethyl acetate partition) was taken and evaporated, weighed 2 mg and dissolved in methanol 200 µL. The mobile phase of the TLC system was hexane: ethyl acetate (3:1) and compound of standard 1'-acetoxy cavasol acetate and trans p-dicumaryl acetate. The elution results were observed at the wavelength of 254 nm.

MTT Assay

The harvested cells were seeded onto 96 well plates with 3000 cells on in each in 100.0 µL culture medium. The cells were then incubated for 24 hours in CO_2 5%, 37^{o}C. 100 µL samples were added then cells were incubated for 24, 48 hours. After incubation, the medium was discarded and cells were washed using PBS pH 7.4 prior to be replaced with 100.0 µL culture medium and added with 15 µL MTT (5 mg/mL) and followed by six hours incubation. The formazan was diluted in 10% SDS HCl (0.01%) for 12 hours. The result was measured using ELISA reader (595 nm).

RESULTS AND DISCUSSION

The extraction conducted by maceration obtained yield from Pasar Legi surakarta, Pasar Beringharjo Yogyakarta and Pasar Wonogiri, in the other were 2.95; 2.92 and 8.23% b/b. According to research Bermawie, *et al.*, 2012, the location of galangal planting in Karanganyar obtained the best weights and rhizomes morphology characteristics compared to planting in Kulonprogo Yogyakarta. Different location of galangal planting effect on the diversity of morphological characters of rhizomes, harvest (harvest time), and quality of chemical content (Bermawie, *et al*., 2012).

Based on TLC profile and Rf value, the extract contained chemical compounds including trans p-coumaril and ACA (Fig. 1).

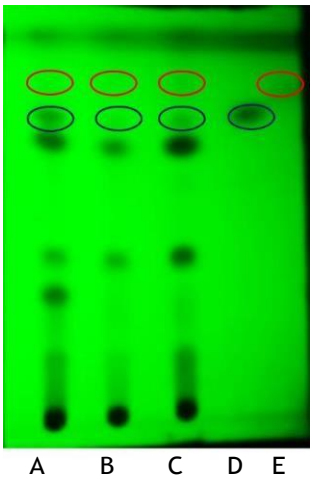

Figure 1. Chromatogram of AGE. AGE from Pasar Legi Surakarta (A); Pasar Wonogiri (B); Pasar Beringharjo Yogyakarta (C); Standard compound J4 / trans-p-Coumaril diasetat (D); and J2/ 1'-acetoxy chavicol asetate (E). Stationary phase = silica gel GF_{254}, mobile phase= hexane : ethyl acetate (3:1). Visualization at UV 254 nm.

Due to structure of ACA, the physical properties of ACA are semipolar, and soluble on ethyl acetate. So that, the sample AGE first performed was partition using ethyl acetate. Intensity of spots from pasar Legi and pasar Beringharjo are more intense than Pasar Wonogiri. This result indicated that concentration of active compound was higher than one. Trans-p-coumaril was used as standard because it is isomer from ACA.

Based on spectrophotometric determination, ACA contained in extract from three local market was different in line with TLC spots. Linierity of ACA obtained from 0.625-10 µg/ mL was good, with regression equation y = 0.139 +0.080 and r square = 0.995. The concentration of ACA in the three local markets, namely Pasar Legi Surakarta, Pasar Beringharjo, Yogyakarta and Pasar Wonogiri were 3.796; 0.035 and 0.009 % w/w. Based on the concentration of ACA, samples from Pasar Legi Surakarta has the highest concentration compared to two others. It was in line with TLC profiles.

Cytotoxic activity

Based on table 1, the interesting data was the high potency of samples from Pasar Legi Surakarta that have IC_{50} value less than 50 µg/mL on all cancer cell lines. That value described that the potency of cytotoxic is great. Due to IC_{50} values of samples from Pasar Legi Surakarta, Pasar Beringharjo Yogyakarta, and Pasar Wonogiri showed the great potential cytotoxic activity with the highest activity was from Pasar Legi Surakarta. It was not surprising, because the highest concentration of ACA was from Pasar Legi Surakarta. Previous research conducted by Chudiwal (2010) stated that ACA has cytotoxic activity on COR L23 cells and MCF7 cells with IC_{50} values of 7.8 µM and 23.9 µM. Samarghandian (2014) stated that ethanol extract of *Alpinia galanga* has cytotoxic activity on MCF7 cells lines after 48 hours incubation resulted in IC_{50} value of 400 ± 11.7 µg/mL. Galangal ethanol extract with concentration of 675 mg/kg bw has anticancer activity and able to inhibit cell proliferation and growth of C3H cell tumor (Asri and Winarko, 2016).

Sample which has an IC_{50} value greater than 100 µg/mL generally considered to have lack cytotoxic activity (Rajabalian, *et al*, 2007). ACA has anticancer activity by apoptotic induction mechanism through activation of the caspase-3 cascade (Asri and Winarko, 2016), suppress initiation and promotion of tumor-induced chemical and viral compounds (Elfahmi, 2006).

CONCLUSION

1. ACA concentration of ACE Pasar Legi Surakarta, Beringharjo Jogja Market, and Wonogiri Market are 3.798; 0.035; and 0.009% w/w.
2. The IC_{50} values of ethanolic extract of AGE Pasar Legi Surakarta, Beringharjo Yogyakarta, and Wonogiri, on HeLa cell were 13.26; 36.32; and > 100 µg/mL.
3. Samples with highest concentration of ACA had great cytotoxic activity.

Table 1. IC_{50} Values of AGE from three local Markets on Various cancer cell lines

No.	Samples	Cancer cell lines	IC_{50} (µg/mL)
1	Pasar Legi Surakarta	T47D	12.50
2		HeLa	13.20
3		MCF7	15.80
4	Pasar Wonogiri	T47D	171.02
5		HeLa	>100
6		MCF7	>100
7	Pasar Beringharjo, Yogyakarta	T47D	33.87
8		HeLa	36.32
9		MCF7	>100

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